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RESEARCH PAPER

A longitudinal assessment of quality stability and edible safety of commercial yogurt beyond the stated expiration date

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Abstract

This study longitudinally assessed the quality stability and edible safety of two commercial yogurt brands stored at 4 °C, 24 °C, and 32 °C for up to 49 days beyond the expiration date. Microbiological, physicochemical, and sensory analyses were conducted to evaluate changes in safety, functionality, and acceptability. Results indicated that both yogurts remained microbiologically safe, with no detection of Enterobacteriaceae, *Salmonella*, or *Staphylococcus aureus*, and yeast/mold counts within regulatory limits (≤ 30 CFU/mL). Lactic acid bacteria count consistently exceeded 1×10^6 CFU/mL. Both products maintained acceptable quality for 2–3 weeks at 4 °C, with sample B demonstrating superior stability in acidity (fluctuating within only 3.8 °T), viscosity (retaining 2680 mPa·s after 49 days), water-holding capacity (maintaining 59.6–72.6%), and sensory attributes (overall score >80 points at 21 days post-expiration). Elevated temperatures accelerated deterioration, particularly in taste, texture, and structural integrity. sample B, containing *Bifidobacterium animalis* subsp. *lactis* and higher protein content, exhibited enhanced resistance to post-expiration quality loss. The findings suggest that yogurt stored at 4 °C may remain edible beyond the labeled date, providing a scientific basis for the dairy industry to consider extending "best-before" periods for certain products and for policymakers to promote the use of differentiated date labels to reduce household food waste.

Keywords

Expiration date, Food safety, Food waste, Microbiological analysis, Physicochemical properties, Quality stability, Sensory evaluation, Shelf-life extension, Yogurt

Introduction

Global food waste poses a critical challenge to sustainable development, economic stability, and food security (Campbell and Feldpausch 2022). This issue is particularly salient for the growing category of fermented and functional foods, such as yogurt, which are valued for their health benefits and are central to industry innovation (Maitha et al. 2021; Giuffrè and Giuffrè 2024). Consumers constitute the largest source of food waste across the entire supply chain, surpassing combined losses from handling, processing, transportation, and storage (Commission for Environmental Cooperation 2017). A significant proportion of this waste, both in retail and household settings, is associated with expiration dates, with an estimated 20% of household waste attributable to products exceeding their labelled shelf life (Garrone et al. 2014; Leib 2023). This behavior is largely driven by consumer perception of expired products as unsafe, leading to precautionary disposal (Tsiros and Heilman 2005; Theotokis et al. 2012).

Dairy products represent the second most wasted category in households, following fresh fruits and vegetables (De Laurentiis et al. 2018). In particular, yogurt—a widely consumed fermented dairy product—is frequently discarded as it approaches or passes its printed date, despite potential edibility (Embrain Trend Monitor 2022).

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A key issue underlying this behavior is the prevalent misinterpretation of expiration labels as strict safety indicators, rather than markers of optimal quality (Newsome et al. 2014; Campbell and Feldpausch 2022). In fact, a growing body of evidence suggests that many foods remain safe and acceptable for consumption beyond their printed dates if stored appropriately (Zielińska et al. 2020). For instance, Leib (2023) noted that—barring storage under abusive conditions—there is no direct evidence linking the consumption of properly stored expired foods to foodborne illness. Regulatory bodies such as the China Food and Drug Administration (2015) also emphasize that expiration dates indicate reduced quality rather than imminent safety risks, particularly in products such as juices, grain-based goods, and dairy items. Empirical studies, including a survey by the Korea Consumer Agency (Zhang 2025), further support that many sealed, refrigerated products—including milk, dairy beverages, and cheese—can maintain safety and quality for days or weeks beyond their labelled dates. Microbiological safety was confirmed for milk, pasta, mayonnaise, and jam through laboratory testing, remaining evident even six months beyond the designated "best before" date (Zielińska et al. 2020).

Despite these insights, a conspicuous research gap remains regarding the systematic assessment of commercial yogurt quality and safety immediately beyond its expiration date. Although yogurt's properties within shelf life are well-documented (Meng et al. 2013; Shin and Lee 2018; Zhang 2023), longitudinal studies on its post-expiration condition are scarce. This lack of empirical evidence leads to inconsistent consumer behavior and inefficient policy frameworks. Therefore, this study aimed to evaluate the potential extension of the minimum durability date for selected durable commercial yogurt through microbiological, physicochemical, and sensory analyses conducted both on and after the manufacturer's designated date, with the intention of generating robust scientific evidence to support consumer education, reduce food waste, and inform future date labelling policies.

Materials and methods

This study aims to empirically evaluate the quality and safety of commercial yogurt under refrigerated conditions for up to several weeks after its expiration date. Specific objectives include:

(1) Monitoring physicochemical changes. To comprehensively capture the multifaceted nature of yogurt deterioration, key physicochemical parameters were selected based on their direct relevance to safety, quality, and consumer acceptance: 1) titratable acidity, a fundamental parameter in fermented dairy products, was monitored as a key indicator of post-fermentation microbial activity and product stability. Driven by ongoing bacterial metabolism, post-acidification can lead to sensory rejection due to excessive sourness, while an atypical decline may signal microbial die-off, thus serving as a critical marker for assessing edibility; 2) water-holding capacity (WHC) and whey separation were chosen as direct measures of structural integrity, the loss of which is a major textural defect; 3) viscosity was tracked as a key rheological property defining mouthfeel; 4) reducing sugar content was monitored as a sensitive indicator of ongoing microbial metabolism, reflecting the dynamic balance between lactose hydrolysis and its subsequent consumption by lactic acid bacteria for energy and acid production. Its dynamics thus provided crucial insights into the residual metabolic activity of the microbiota and the potential for spoilage.; 5) the assessment of antioxidant activity served two primary purposes: to evaluate the stability of functional bioactive compounds post-fermentation, and to gauge the product's functional integrity beyond its expiration date. A decline in this activity indicates oxidative deterioration and a consequent loss of functional quality, offering insight into value retention that extends beyond safety considerations; and 6) color and turbidity were employed as objective indicators of visual appearance and colloidal stability, respectively. Turbidity was employed as a sensitive, rapid indicator of colloidal stability. Changes in turbidity can signal early-stage structural breakdown, such as protein aggregation, whey separation, or microbial growth, which precede visible syneresis. This allows for the early detection of physical instability in the product matrix.

(2) Assessing microbiological dynamics, including total count of yeast and moulds, Enterobacteriaceae, lactic acid bacteria (LAB) viability and growth of pathogenic microorganisms.

(3) Evaluating sensory attributes over time (Fig. 1). We hypothesize that yogurt stored consistently at 4 °C will experience gradual sensory deterioration without significant compromise to safety in the short-term post-expiration.

Samples preparation

Two commercially available yogurt brands were selected and purchased in December, 2024 for this study: Brand A (Mengniu), representing a leading national dairy producer, and Brand B (Wenshi), representing a prominent regional dairy manufacturer in China. All samples were produced in compliance with manufacturing licensing requirements and adhered to standardized hygiene production standards. The details of the samples are shown in Table 1.

The purchased yogurt samples were initially stored at 4 °C until the expiration date. Subsequently, they were transferred to and maintained at three different temperature conditions: 4 °C (refrigeration), 24 °C (room temperature), and 32 °C (elevated temperature) for further

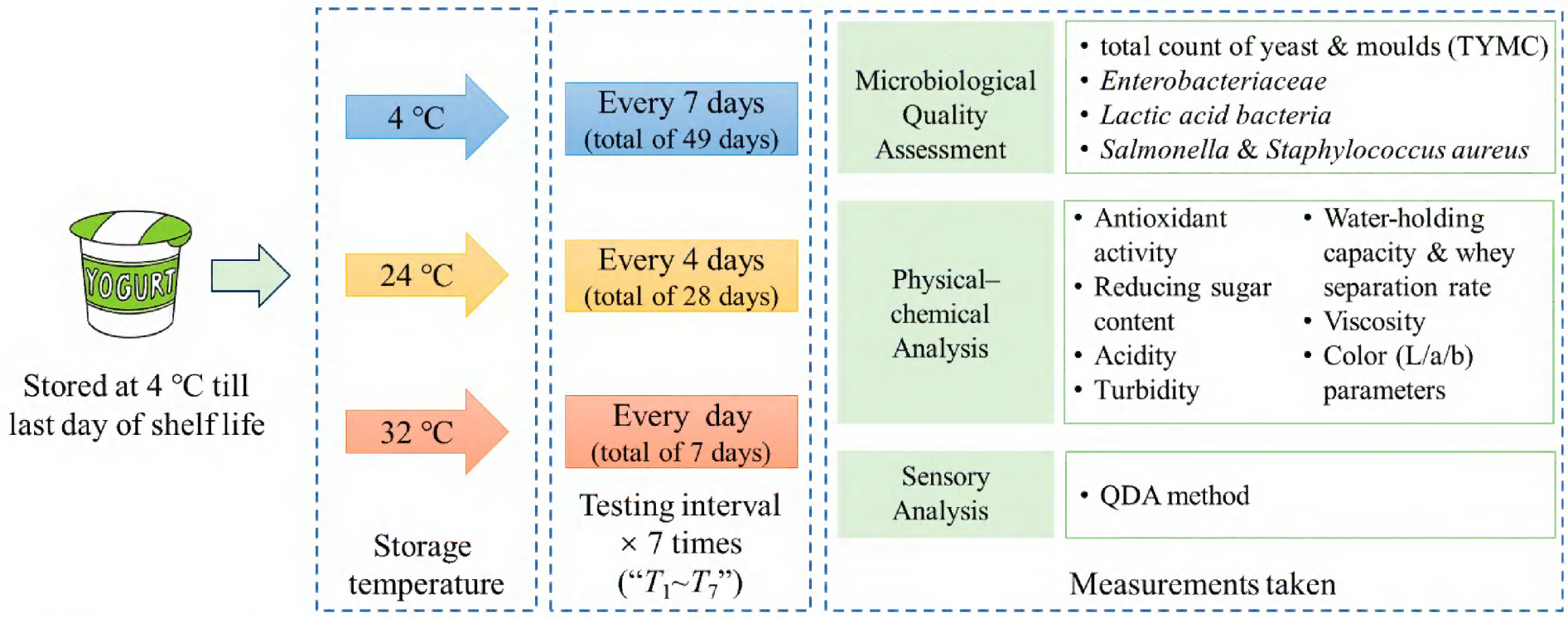


Figure 1. Study outline.

Table 1. Raw material information of the two yogurt brands used in the experiment.

Brand	Nutrition Facts (per 100 g)	Ingredients
A. Wenshi Plain Original Yogurt	Energy 358 kJ, Protein 2.4 g, Fat 3.4 g, Carbohydrate 11.5 g, Sodium 60 mg, Calcium 85 mg	Raw milk (≥80%), White sugar, *Streptococcus salivarius* subsp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, Gelatin, Acetylated distarch phosphate, Mono- and diglycerides of fatty acids
B. Mengniu Fresh Daily Plain Fermented Milk	Energy 405 kJ, Protein 3.3 g, Fat 4.2 g, Carbohydrate 11.4 g, Sodium 60 mg, Calcium 100 mg	Raw milk, White sugar, Milk protein powder, *Bifidobacterium animalis* subsp. *lactis*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*

Note: Information from labels of product packaging.

storage until analysis. Sampling was performed at fixed intervals: every 7 days for the 4 °C group where T_n refers to 7 × n days after the expiration date, every 4 days for the 24 °C group where T_n refers to 4 × n days after the expiration date, every 4 days, and every 1 day for the 32 °C group where T_n refers to 1 × n days after the expiration date. At each scheduled time point, three replicate samples were taken from each temperature group for subsequent analysis. The total duration of the experiment was 49 days.

With two brands, three storage temperatures, multiple time points (up to 7 per temperature), and three replicates per condition, the total number of individual samples analyzed exceeded 300, providing robust data for statistical analysis.

Microbiological quality assessment

The microbiological analyses were conducted according to standard methods. The number of bacteria from the Enterobacteriaceae family was performed using the colony-count technique as specified in ISO 4832:2006. Moulds and yeasts were quantified according to ISO 21527-1:2008. LAB were enumerated following the Chinese National Standard GB 4789.35—2016. Detection of *Salmonella* spp. was carried out based on the horizontal method described in ISO 6579-1:2017. Enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) was conducted in accordance with ISO 6888-1:2021, Part 1 (Method using Baird-Parker agar medium).

Physico–chemical analysis

Antioxidant activity

The DPPH free radical scavenging assay was performed according to the method described by Brand-Williams et al. (1995) with minor modifications. Briefly, 2 mL of the sample extract was mixed with 2 mL of 0.2 mmol/L DPPH solution. The mixture was incubated for 30 minutes at room temperature in the dark, followed by centrifugation at 4000 r/min for 10 minutes. The absorbance of the supernatant was subsequently measured at 517 nm.

Reducing sugar content

The reducing sugar content was measured following the reference method specified in ISO 8069:2005.

Acidity

The titratable acidity of the yogurt samples was determined according to the Chinese National Standard GB 5009.239-2016. Briefly, samples were diluted with deionized water in a 1:4 (*v*/*v*) ratio and titrated with 0.1 M sodium hydroxide (NaOH) solution using phenolphthalein as the pH indicator. Acidity was expressed in Thorner degrees (°T), defined as the volume (mL) of 0.1 M NaOH required to neutralize acids present in 100 mL of yogurt.

Turbidity

The turbidity of the yogurt samples was determined according to the method described by Choi et al. (2020). with minor modifications. Briefly, each sample was homogenized

by gentle shaking, and 200 μL of yogurt was accurately pipetted and diluted with deionized water to a final volume of 10 mL. The absorbance of the diluted samples was then measured at a wavelength of 860 nm.

Water-Holding Capacity (WHC)

Water-holding capacity was assessed using a centrifugation-based method. Precisely 10 g of yogurt (W_1) was centrifuged at 4000 r/min for 20 min at 4 °C using a high-speed refrigerated centrifuge (TGL-16M, Hunan Xiangyi Laboratory Instrument Development Co., Ltd., China). After centrifugation, the supernatant was carefully removed and weighed (W_2). The WHC was calculated as follows:

$$\mathrm{WHC\%} = (W_1 - W_2)/W_1 \times 100 \quad (1)$$

Where W_1 represents the initial sample weight and W_2 the weight of the expelled supernatant.

Whey separation rate

Whey separation rate was determined according to the method described by Sun et al. (2020) with slight modifications. Briefly, 10 g of yogurt sample was evenly placed on a pre-weighed filter paper (Whatman No. 1) supported by a funnel mounted on a graduated beaker. The assembly was allowed to stand at ambient temperature (25 °C) for 100 minutes to facilitate natural whey drainage. The whey collected in the beaker was weighed, and the whey separation rate was calculated using the following formula:

$$\text{Whey separation rate\%} = W_{\text{whey}}/W_{\text{initial}} \times 100 \quad (2)$$

Where W_{whey} is the weight (g) of the drained whey and W_{initial} is the initial weight of the yogurt sample (10 g).

Viscosity

The viscosity of yogurt samples was measured using an NDJ-1S digital rotary viscometer (Shanghai Yixin Scientific Instrument Co., Ltd., China) equipped with a No. 3 rotor. Measurements were conducted room temperature of 18 °C with a rotational speed of 6 rpm. After three dynamic measurements under consistent conditions, the mean value was recorded for each sample.

Color

Color parameters were evaluated using a LS173 colorimeter (Shenzhen Linshang Technology Co., Ltd, China) based on the CIE $L^*a^*b^*$ color system. The instrument was calibrated with *a* white standard tile before use. Three replicate measurements were performed for each sample under consistent lighting conditions. The total color difference (ΔE^*) was calculated using the following formula:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

Where ΔL^*, Δa^*, and Δb^* represent the differences in lightness, red–green coordinate, and yellow–blue coordinate, respectively, between the expired sample and the fresh reference sample.

Sensory analysis

Sensory evaluation was performed following the methodology established by Lu et al. (2020). The panel consisted of eight trained assessors (4 males and 4 females, age = 21 years old) who had received professional training to accurately identify sensory attributes of yogurt and were proficient in applying the relevant evaluation criteria. Prior to the experiment, ethical approval was obtained from the institutional review board, and informed consent was acquired from all participants in accordance with ethical guidelines for food sensory studies. To minimize external influences on sensory perception, panelists were instructed to refrain from smoking, consuming alcohol, or ingesting spicy or irritant foods for 12 hours preceding the evaluation.

Panelists evaluated the yogurt based on four sensory attributes: color (milky whiteness, 20 points), aroma (characteristic yogurt fragrance, 20 points), taste (sweetness and sourness balance, 30 points), and texture (smoothness, 30 points), with overall acceptability scored out of a maximum of 100 points. Each attribute was rated on a hedonic scale, where higher scores indicated superior quality. For safety reasons, participants were instructed to taste samples without swallowing and to rinse their mouths thoroughly with water between evaluations. Sensory analysis was conducted only at the third measurement point (T_3) as a representative time to balance the need for sensory data with ethical considerations regarding panelist safety when tasting products significantly beyond their expiration date. This time point effectively captured the differential impact of the three storage temperatures on sensory attributes while minimizing potential risks associated with repeated sampling of expired products.

Data analysis and processing

The experimental data were analyzed primarily by one-way analysis of variance (ANOVA) using SPSS software (version 22; IBM Corp., Armonk, NY, USA). Differences between individual sample means were assessed using Duncan's multiple range test at a significance level of $p \leq 0.05$. Due to the longitudinal nature of the data and the clarity of trends across multiple time points, statistical significance is explicitly described in the "Results and Discussion" text for key comparisons, rather than being denoted on the figures themselves to maintain visual clarity.

Results and discussion

Microbiological quality assessment

Throughout the experiment, no Enterobacteriaceae were detected. Although molds and yeasts were present (Table 2), no significant difference ($p > 0.05$) in mold and yeast counts across storage temperatures and their counts did not exceed the Chinese National Food Safety Standard GB 19302-2010 limit of ≤ 30 CFU/g (mL) for fermented

milk. Additionally, in line with this standard, no *Salmonella* or *Staphylococcus aureus* was detected in any sample, confirming all products were compliant. Our results align with the broader observations of Zielińska et al. (2020), who reported that many sealed, refrigerated dairy products, including yogurt, remained microbiologically safe for months beyond their 'best before' date, with no pathogen detection. This consistency across studies reinforces the notion that properly manufactured and stored yogurt possesses inherent microbiological stability that extends beyond the labeled date.

Table 2. Trends of molds and yeasts variation over time in expired yogurt under different storage temperature conditions (CFU/mL).

Time points	4 °C				24 °C				32 °C			
	mold		yeast		mold		yeast		mold		yeast	
	A	B	A	B	A	B	A	B	A	B	A	B
T_1	1	0	0	1	0	0	0	0	0	0	0	0
T_2	0	0	0	0	1	0	0	0	0	1	0	1
T_3	0	0	1	3	0	1	0	2	0	1	0	3
T_4	1	0	2	3	2	1	1	0	2	0	0	1
T_5	2	2	0	0	1	1	0	1	0	1	0	0
T_6	1	1	0	3	2	0	3	0	1	0	0	0
T_7	3	2	1	11	0	1	2	7	1	2	2	1

According to the Chinese National Food Safety Standard GB 19302-2010 for fermented milk, which requires a LAB count of ≥ 1×10^6 CFU/g (mL), all samples under various test conditions throughout the experimental period were found to comply with this standard.

As illustrated in Fig. 2, lactic acid bacteria (LAB) count in expired fermented milk products displayed complex, multiphasic dynamics across different storage temperatures (4 °C, 24 °C, and 32 °C) over a 49-day period. An initial increase in LAB was commonly observed under most conditions, likely supported by residual nutrients that facilitated continued metabolic activity and proliferation. Subsequent fluctuations, including recoveries in bacterial counts, may be attributed to metabolic adaptation among resistant strains, ongoing proteolytic activity releasing additional nutrients, or spatial heterogeneity within the product enabling localized regrowth. Such multiphasic growth-decline patterns align with earlier observations, such as those of Meng et al. (2013), who reported fluctuating microbial counts in commercial yogurts. However, the longitudinal design employed here over 49 days offers a more detailed resolution of these post-expiration dynamics. Notably, the cyclical LAB patterns observed at 4 °C suggest successive waves of nutrient depletion and microbial adaptation—a phenomenon that has been less documented in earlier studies focused primarily on conventional shelf-life periods.

Specifically, at 4 °C, LAB counts in both samples showed significant fluctuations ($p < 0.05$). For sample B, the count at T_5 (35 days) was significantly higher than at T_1 (7 days), but subsequently declined significantly by T_7 (49 days). This recurring pattern of significant increase followed by a significant decrease ($p < 0.05$) potentially reflects cycles of nutrient depletion and metabolic regeneration. These dynamics suggest repeated phases of stress and recovery, likely mediated by microbial succession or shifts in enzymatic activity (Meng et al. 2013). At 24 °C, LAB did not increase until day 24, likely due to reduced metabolic and enzymatic activity at suboptimal temperatures, necessitating an extended adaptation phase for synthesis of stress-response proteins (Varmanen and Savijoki 2011). At 32 °C, a shorter growth phase was observed, accompanied by a consistent increase in bacterial population.

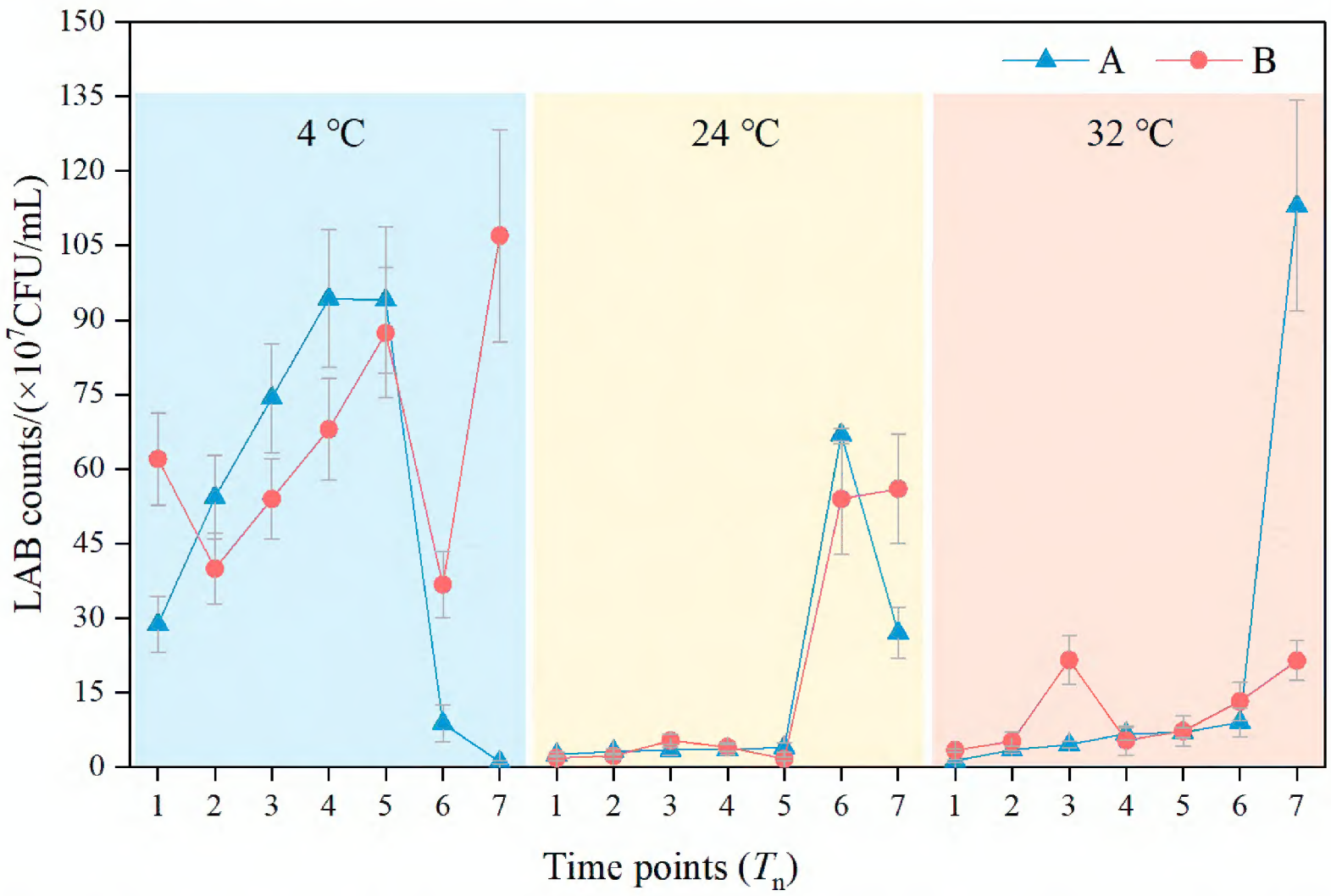


Figure 2. Trends of LAB variation over time in expired yogurt under different storage temperature conditions.

The distinct dynamics of LAB between sample A and B, as illustrated in Fig. 2, are indicative of their different microbial compositions and have direct implications for microbiological safety. The stable and moderated growth of LAB in sample B can be directly attributed to its inclusion of *Bifidobacterium animalis* subsp. *lactis* as a probiotic adjunct culture, in addition to the standard yogurt starters. Bifidobacterium strains are known to produce antimicrobial compounds, such as bacteriocins and organic acids (acetate and lactate), creating an environment that is less conducive to the survival and proliferation of pathogenic and spoilage organisms (Tindjau et al. 2023). This enhanced "competitive exclusion" and "microbial interference" effect, conferred by the specific probiotic strain, likely contributed to the consistent absence of Enterobacteriaceae, *Salmonella*, and *Staphylococcus aureus* in sample B across all storage conditions, thereby reinforcing its superior microbiological safety profile beyond the expiration date compared to sample A.

In contrast, sample A, containing only the classic yogurt starters *Streptococcus salivarius* subsp. *thermophiles* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, exhibited a more volatile LAB population. The symbiotic relationship between *S. thermophilus* and *L. bulgaricus* is highly effective for rapid acid production during fermentation but may lead to a less stable and more self-limiting ecosystem during extended storage, as their primary metabolic focus is lactose fermentation to lactate. While these strains are known for rapid acid production and synergistic growth at elevated temperatures (Settachaimongkon et al. 2014), their ecosystem demonstrated less stability during extended storage. The fluctuating LAB counts suggest a less regulated post-acidification process and a potential lack of the stabilizing or protective metabolites produced by adjunct probiotics. This ecological niche, characterized by greater population volatility and a narrower range of antimicrobial activity, might theoretically create transient opportunities for secondary contaminants if the initial microbial or physical barrier were compromised, although this was not observed in the present study. Although no pathogens were detected in either sample, the comparative instability of sample A's microbiota and its reliance on a less diverse starter culture underscores the enhanced safety assurance and ecological robustness conferred by the specific probiotic strain in sample B.

Physico–chemical analysis

Antioxidant activity

The determination of DPPH radical scavenging activity in expired yogurt serves as an indicator of residual antioxidant capacity, reflecting the stability of bioactive compounds and the extent of oxidative deterioration. This measurement provides supplementary insight into functional preservation and overall degradation status beyond microbial safety assessments. The DPPH radical scavenging activity of yogurt samples was evaluated under three storage temperatures (Fig. 3). The results indicate that both temperature and sample composition significantly influenced antioxidant retention. sample B consistently demonstrated higher DPPH radical scavenging rates across all storage temperatures compared to sample A, with initial values of 62.91% and 54.88%, respectively, further supporting its superior retention of antioxidants such as peptides, exopolysaccharides, and other bioactive metabolites (Martins et al. 2021). Over time, samples stored at 4 °C exhibited a noticeable and statistically significant decline in activity ($p < 0.05$); for instance, sample B decreased significantly from 62.91% to 51.69%, and sample A from 54.88% to 48.32%, likely due to the gradual degradation

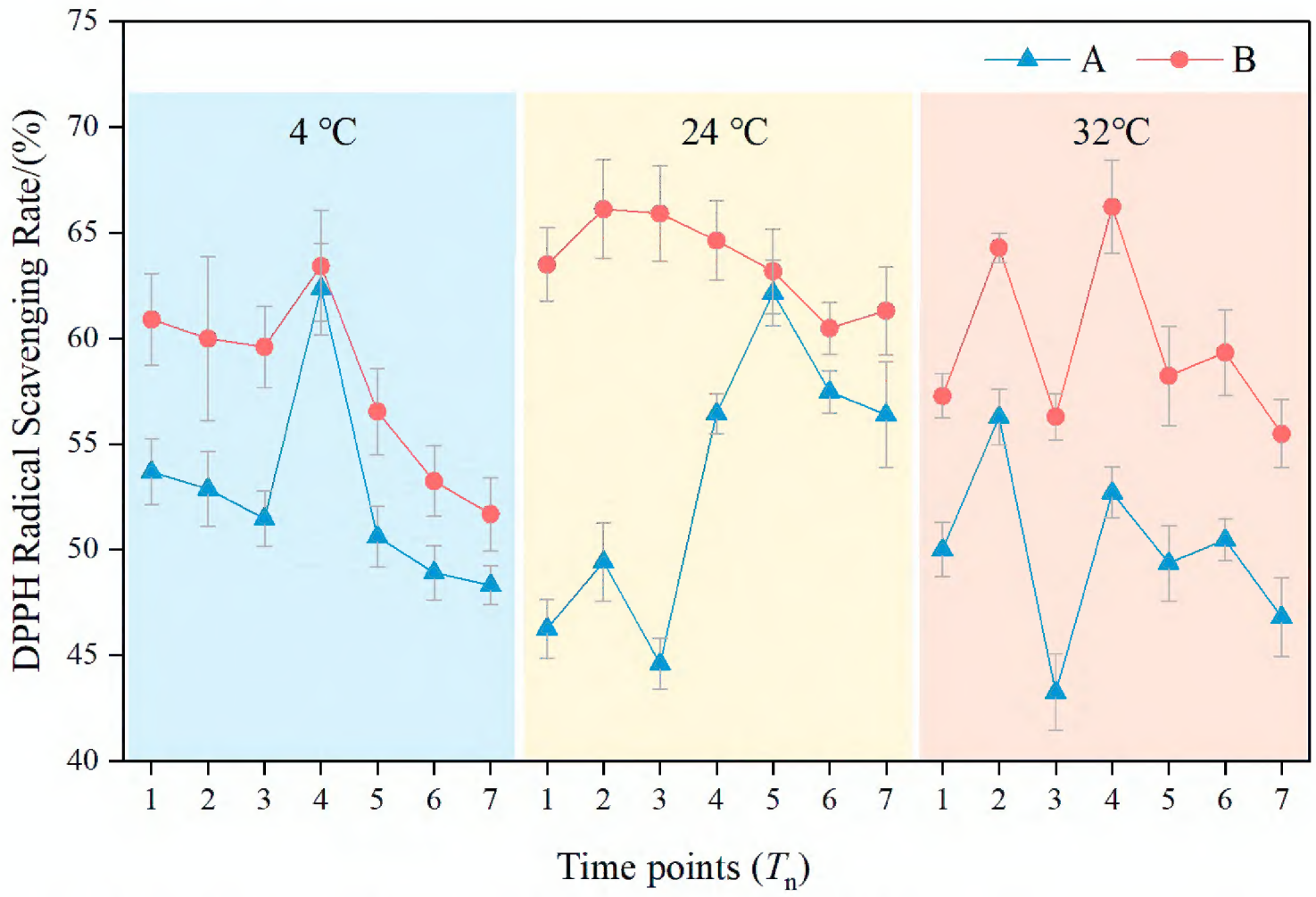


Figure 3. Trend of DPPH radical scavenging rate (%) in expired yogurt under different storage temperatures over time.

of thermolabile antioxidants and microbial consumption of bioactive compounds. In contrast, both 24 °C and 32 °C storage conditions elicited a multiphasic response characterized by an initial significant increase ($p < 0.05$)—as seen in sample B at 24 °C (reaching 66.12% by T_2) and 32 °C (peaking at 66.23% at T_4)—followed by a subsequent significant decrease ($p < 0.05$), suggesting transient microbial metabolic activation and release of antioxidants prior to eventual degradation. Notably, the overall higher scavenging activity observed in sample B, particularly under elevated temperatures, may be attributed to its greater initial antioxidant capacity and enhanced microbial resilience facilitating sustained metabolite release.

Reducing sugar content

The variation in reducing sugar content serves as a sensitive indicator for revealing the microbial metabolic status, residual enzyme activity, and extent of chemical deterioration in expired yogurt. Its dynamics reflect whether the product is undergoing slow decline, remains stable, or is experiencing accelerated spoilage, thereby providing a scientific basis for assessing edibility risks and residual value (Son et al. 2023). The reducing sugar content in yogurt samples stored at different temperatures showed distinct dynamic trends over time (Fig. 4). At 4 °C, the reducing sugar content remained relatively stable throughout the storage period, with no significant differences ($p > 0.05$) between most time points for either sample. This indicates minimal microbial or enzymatic activity, consistent with suppressed metabolic processes under refrigeration. Substantial decreases were observed at elevated temperatures.

The initial increase followed by a subsequent decrease in reducing sugar content observed in both samples at 4 °C and 24 °C likely results from the dynamic balance between enzymatic release and microbial consumption of sugars, driven by temperature-dependent metabolic activities. The biphasic trend suggests that sugar dynamics in expired yogurt are governed by two competing processes: early-stage sugar liberation via enzymatic hydrolysis and autolysis, and late-stage sugar consumption by microbial metabolism. Temperature modulates the rate and extent of these processes, with higher temperatures accelerating both phases. This pattern highlights the complex biochemical interactions that continue to occur even under suboptimal storage conditions. Specifically, samples stored at 32 °C exhibited the most pronounced significant reduction ($p < 0.05$) in reducing sugar levels, where reducing sugar levels dropped markedly over time. This accelerated decline can be attributed to enhanced microbial metabolic activity and enzymatic reactions at elevated temperatures, promoting the utilization of sugars for energy production and acid formation.

The divergence in sugar consumption rates between temperatures highlights the strong dependence of microbial activity on storage conditions. The rapid reduction at higher temperatures may correlate with accelerated spoilage, while the stability at 4 °C reflects better preservation of initial composition. These results underscore the critical role of temperature control in maintaining yogurt quality and extending shelf life by limiting microbial degradation of carbohydrates.

Acidity

The titratable acidity of yogurt samples stored at different temperatures exhibited distinct trends over time (Fig. 5), reflecting complex biochemical and microbial dynamics during storage. Sample A, with an initial acidity of 81.30 °T, consistently demonstrated higher titratable acidity at elevated temperatures—reaching 146.47 °T at 24 °C and 221.67 °T at 32 °C by the end of storage—compared to sample B (initial: 90.07 °T), which remained

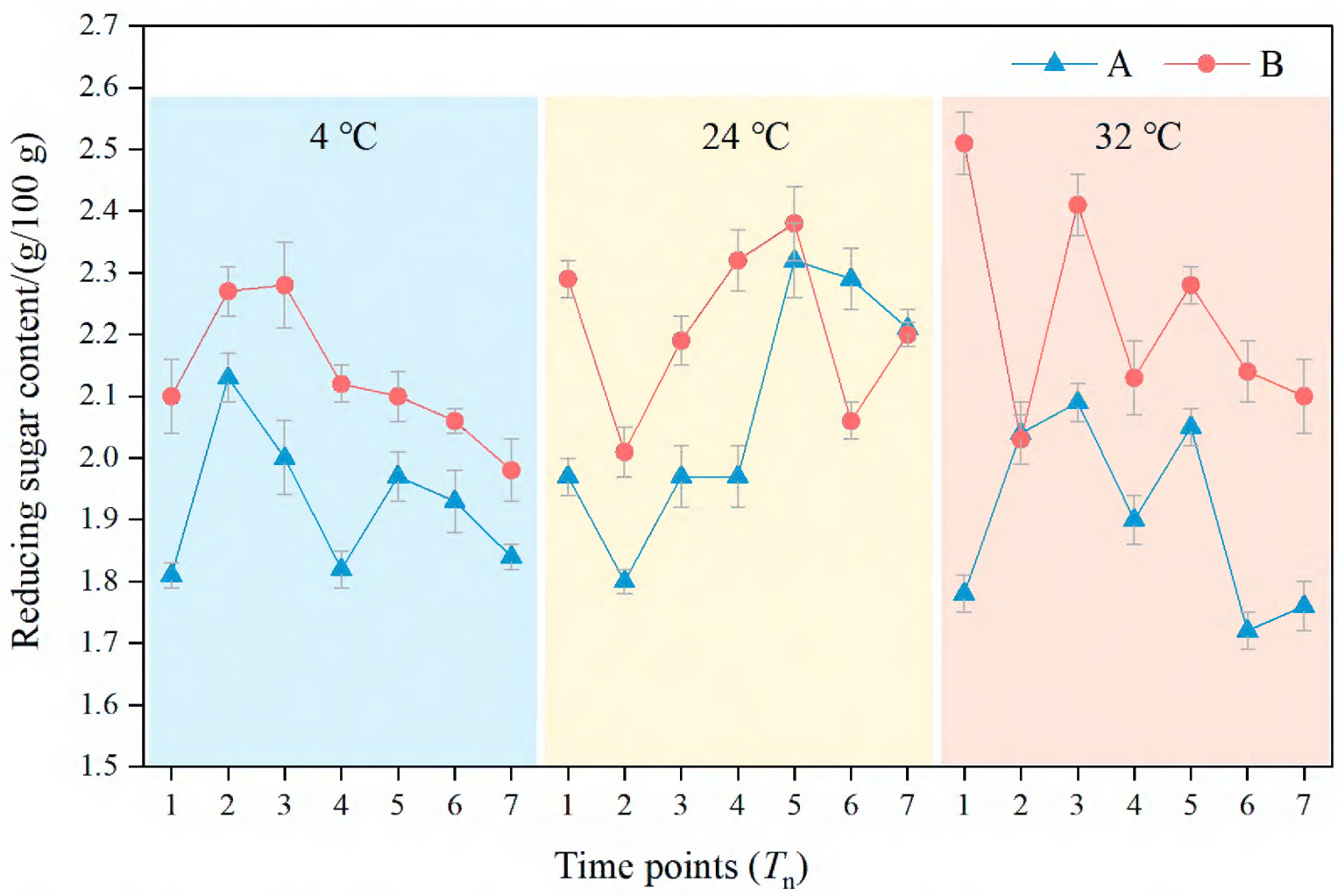


Figure 4. Trend of reducing sugar content (g/100 g) in expired yogurt under different storage temperatures over time.

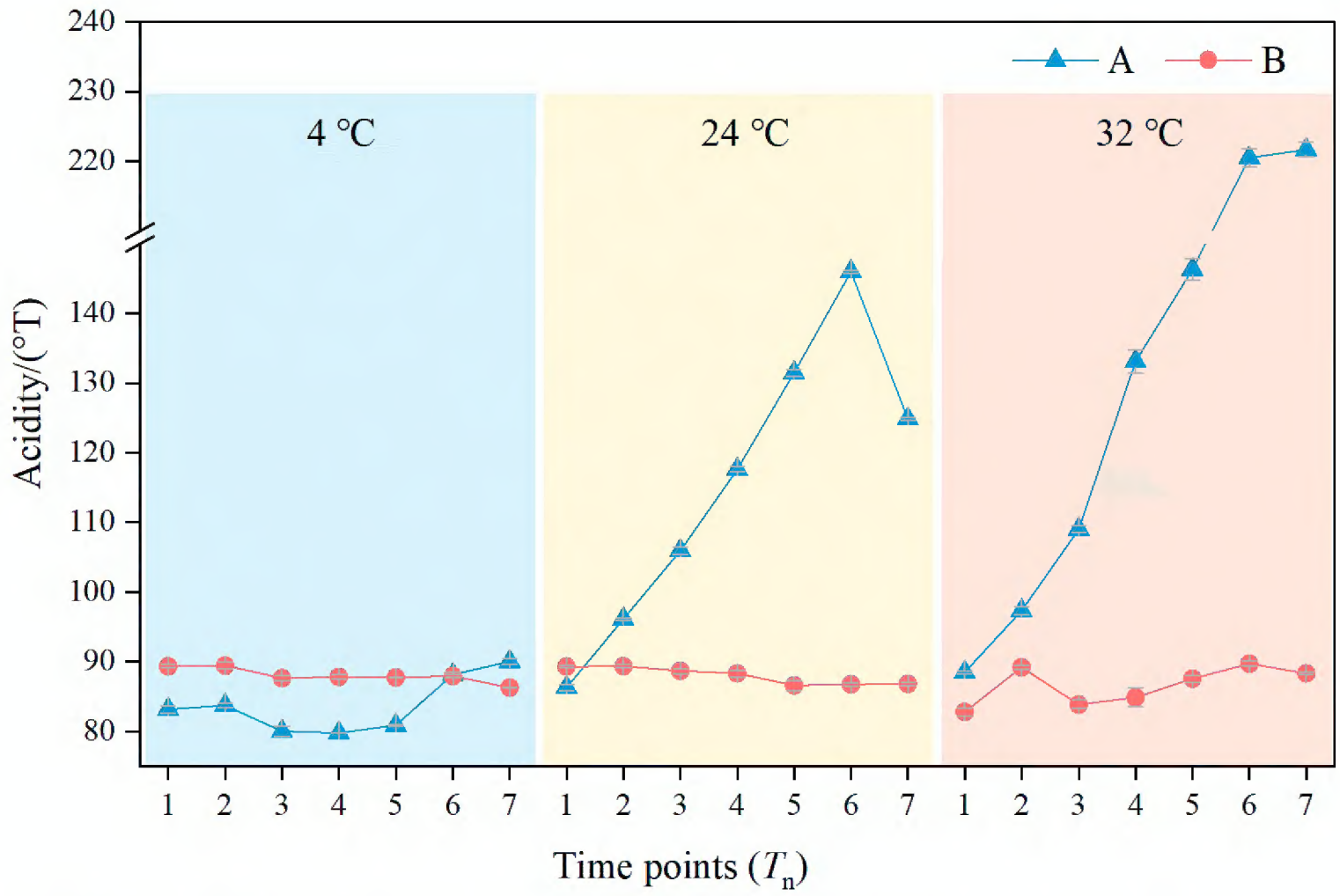


Figure 5. Trend of acidity (°T) in expired yogurt under different storage temperatures over time.

stable within a narrow range (e.g., 86.27–89.46 °T across all conditions). In contrast, at 4 °C, both samples maintained relatively stable acidity, with sample A fluctuating between 79.78–90.03 °T and sample B between 86.27–89.46 °T.

The pronounced increase in titratable acidity observed in sample A at 24 °C and 32 °C was statistically significant ($p < 0.05$ at most time points compared to the initial value). For instance, at 32 °C, the acidity at T7 (221.67 °T) was significantly higher ($p < 0.05$) than at all preceding measurement points, confirming a relentless post-acidification process. Compared to its stability at 4 °C, these changes can be attributed to the combined effects of its composition and microbial ecology. The high proportion of raw milk and added white sugar provide abundant fermentable substrates (lactose and sucrose), which are readily utilized by the classic yogurt starter cultures—*Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. These strains exhibit strongly thermophilic characteristics, with metabolic and enzymatic activities significantly enhanced at higher temperatures, accelerating glycolysis and lactic acid production. While stabilizers such as gelatin, acetylated distarch phosphate, and mono- and diglycerides of fatty acids improve textural stability, they do not exert significant buffering effects against acid accumulation. At 4 °C, the metabolic activity of both bacterial strains is markedly suppressed, leading to minimal acid production and thus stable acidity. In contrast, at 24 °C and especially at 32 °C, the synergy between *S. thermophilus* (rapid initial acidification) and *L. bulgaricus* (proteolytic activity releasing peptides and amino acids that further stimulate growth) drives sustained post-acidification, resulting in a sharp and continuous increase in titratable acidity over time.

The exceptional stability of titratable acidity in sample B across time and temperature conditions is likely attributable to its unique compositional and microbial profile. The addition of milk protein powder may enhance buffering capacity (Marafon et al. 2011), while the critical differentiating factor is the presence of *Bifidobacterium animalis* subsp. *lactis*. This finding is supported by Son et al. (2023), who demonstrated that yogurts fortified with Bifidobacterium strains exhibited moderated post-acidification during storage. This probiotic strain modulates the overall acid production profile through its distinct "bifid shunt" metabolic pathway, which yields acetate and lactate in a specific ratio (approximately 3:2), unlike the homolactic fermentation dominated by lactate in classic starters. This production of acetate, a potent antimicrobial, not only contributes to microbiological safety but also competitively can inhibit the more aggressive acid-producing activity of conventional yogurt strains (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) (Li et al. 2019; Ma et al. 2025). Thus, the microbial interplay in sample B creates a more balanced and self-regulating metabolic equilibrium, which helps maintain a stable environment that may competitively inhibit spoilage organisms, thereby supporting ongoing microbiological safety. This, combined with potentially reduced proteolytic activity and limited sugar utilization efficiency, results in a system that minimizes post-acidification and maintains acidity within a narrow range despite external storage variations (≤ 3.8 °T across all temperatures).

Stability

Turbidity serves as a sensitive indicator of colloidal stability in yogurt, reflecting integrated changes in the protein-lipid system and microbial activity (Aryana and Olson 2017). It enables early prediction of defects such as whey separation and ensures product consistency (Miyaji et al. 2020). As illustrated in Fig. 6, turbidity measurements

under different storage temperatures revealed that sample B (initial value: 0.502) exhibited notably greater colloidal and structural stability than sample A (initial value: 0.648). Sample B maintained consistently lower and more stable turbidity values across all conditions, e.g., ranging from 0.379 to 0.674, which can be attributed to its higher protein content (3.3 g/100 g vs. 2.4 g/100 g in sample A). This observation corroborates the findings of Delikanli and Ozcan (2016), who reported that fortification with milk proteins led to a denser protein network and improved textural properties in yogurt. Furthermore, the potential role of expolysaccharides (EPS) in stabilizing the colloidal system cannot be overlooked. While our study did not directly quantify EPS, the stable viscosity and WHC of Sample B, especially at 4 °C, are consistent with the rheological improvements attributed to EPS-producing cultures in other works (Bullard et al. 2018; Wang et al. 2024). In contrast, the significant viscosity loss and structural vulnerability of Sample A at elevated temperatures highlight the formulation-dependent nature of quality decay, a factor that should be central to any discussion on shelf-life extension.

In contrast, sample A showed greater variability and a significant decline in turbidity under 4 °C storage, decreasing from an initial value of 0.648 to 0.668 by day 7 ($p < 0.05$), with fluctuations between 0.685 and 1.032 throughout the period. This suggests cold-induced structural reorganization, including gel contraction, syneresis, and localized aggregation of casein and fat globules, leading to reduced light scattering and increased microscopic heterogeneity (Elmarhoum and Ako 2021; Lorenzen et al. 2024). By comparison, elevated temperatures helped maintain system homogeneity in both samples, as seen in sample A's relatively stable profiles at 24 °C (0.665–1.063) and 32 °C (0.506–0.725), through sustained microbial metabolism and enzymatic activity that support EPS production and colloidal stabilization (Brennan and Tudorica 2008).

The water-holding capacity (WHC) and whey separation rate of sample A and B under different storage temperatures and over time are shown in Fig. 7. Sample A, with an initial WHC of 70.73%, exhibited significantly lower WHC throughout storage (e.g., declining to 51.30% at 4 °C, 55.58% at 24 °C, and 50.78% at 32 °C by day 7) and a correspondingly higher whey separation rate ($p < 0.05$), indicating a comparatively less stable gel matrix. In contrast, sample B demonstrated superior stability, maintaining high WHC (initial: 72.91%; ranging from 59.63% to 72.59% across temperatures) and lower whey separation (initial: 13.43%; remaining below 25.57% in all conditions).

Throughout the storage period, the WHC of both samples remained largely stable at 4 °C, with sample B showing minimal fluctuation (60.17–61.94%) compared to sample A (51.30–68.85%). Similarly, the whey separation rate showed limited variation at low temperatures. However, a noticeable increasing trend in whey separation was observed in sample B at 24 °C (rising from 12.59% to 25.57%) and 32 °C (from 15.94% to 21.45%), suggesting temperature-dependent structural weakening despite its overall stability. sample A consistently exhibited higher syneresis across all temperatures, reinforcing its structural vulnerability. These results highlight the critical role of composition and storage temperature in determining yogurt's physical stability, with sample B's formulation—potentially enhanced by higher protein content or stabilizers—providing greater resistance to post-expiration deterioration.

Above all, sample B demonstrated consistently higher stability across temperatures and over time after expiration, maintaining strong colloidal integrity and minimal

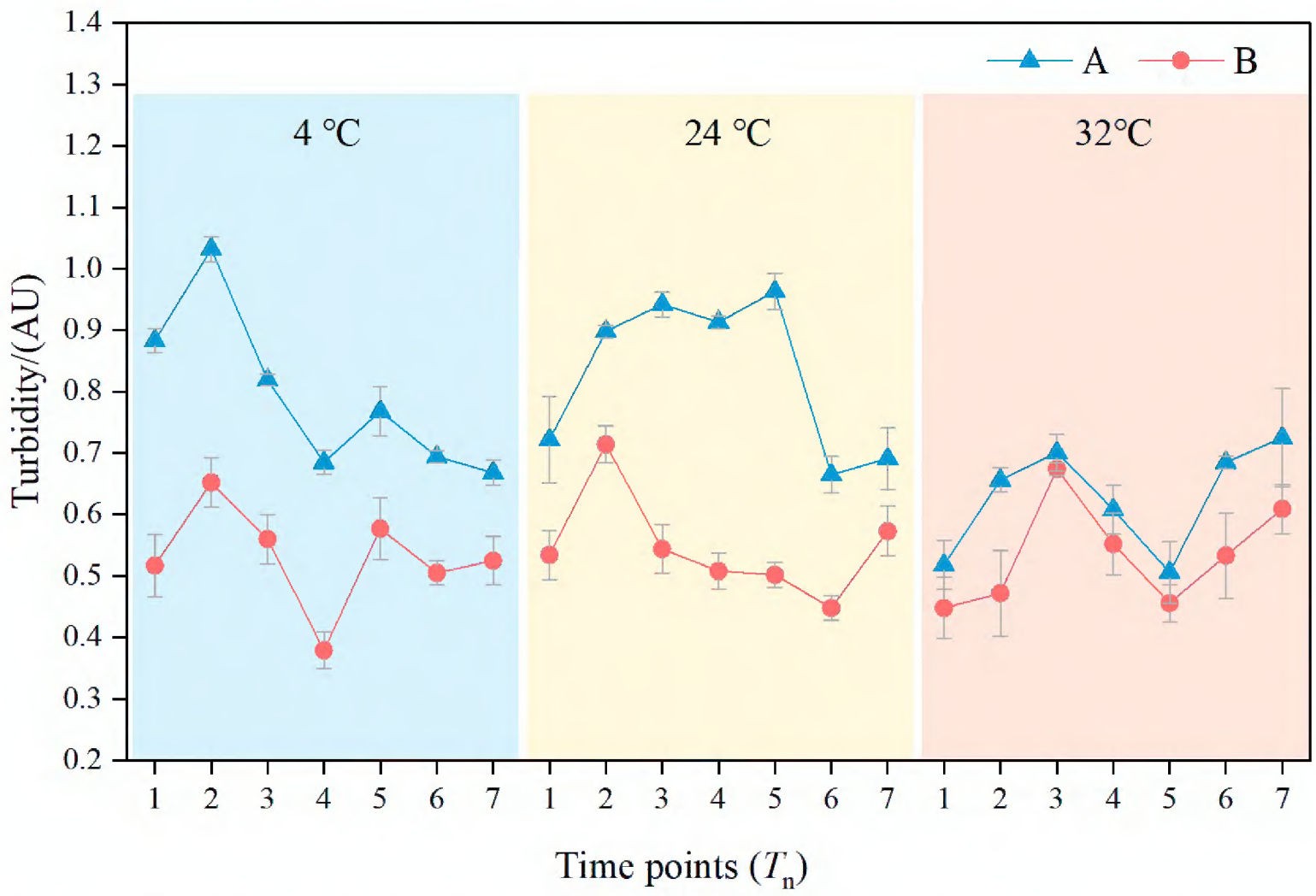
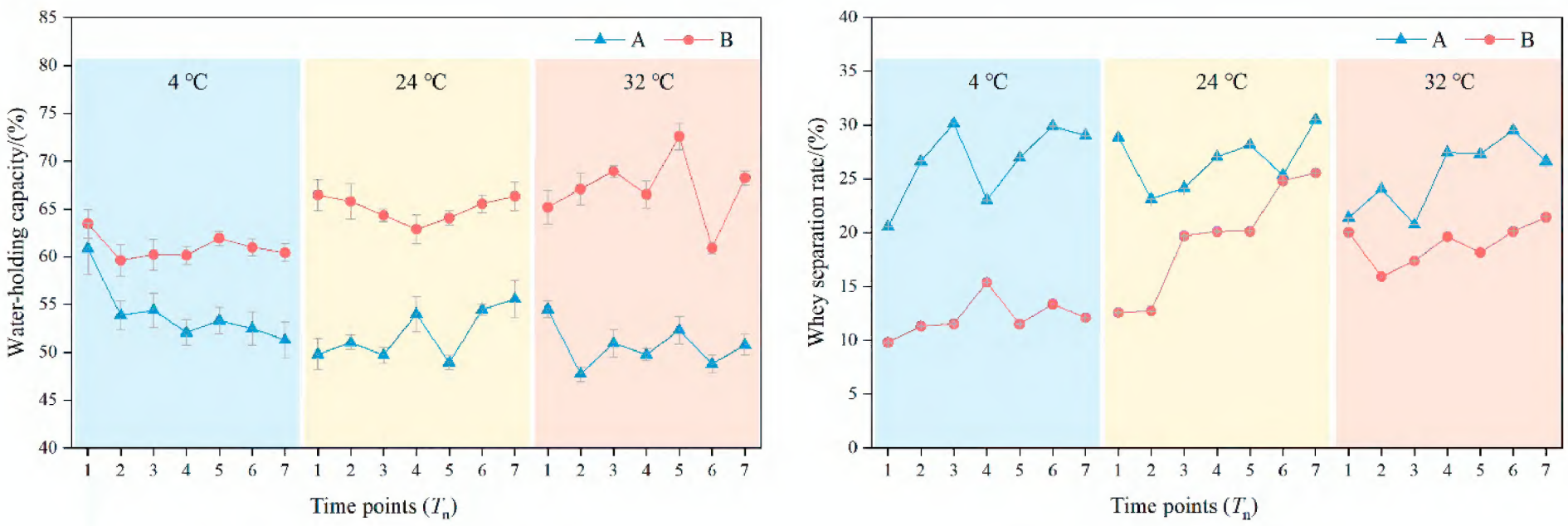


Figure 6. Trend of turbidity (AU) in expired yogurt under different storage temperatures over time.



(a) Water-holding capacity (b) Whey separation rate

Figure 7. Trend of water-holding capacity (a) and whey separation rate (b) in expired yogurt under different storage temperatures over time.

structural change due to its higher protein content and potential microbial or stabilizer enhancements. In contrast, sample A showed significant sensitivity to temperature, with pronounced degradation at 4 °C due to cold-induced syneresis and structural heterogeneity, though it remained relatively stable at elevated temperatures. Both samples retained largely stable water-holding capacity over time, but sample B exhibited better overall resistance to whey separation, particularly under non-refrigerated conditions.

Viscosity

The viscosity measurements revealed distinct degradation patterns influenced by both storage temperature and sample composition (Fig. 8). At 4 °C, the viscosity of both samples remained relatively stable with minor fluctuations, indicating preserved gel structure due to suppressed microbial and enzymatic activity. Sample A (initial: 2580.3 mPa·s) exhibited a more pronounced and statistically significant decline ($p < 0.05$), decreasing from 2265.3 to 1023.9 mPa·s (54.8% reduction), indicating greater structural vulnerability. In contrast, sample B (initial: 6487.0 mPa·s) demonstrated better retention, declining from 4221.3 to 2680 mPa·s (36.5% reduction), with the reduction at 4 °C being statistically significant ($p < 0.05$) but proportionally smaller.

Samples stored at 24 °C and 32 °C exhibited significant viscosity reduction ($p < 0.05$), suggesting progressive breakdown of the protein network and loss of structural integrity. At 24 °C, sample B showed accelerated degradation, with viscosity dropping from 4203.7 to 982.9 mPa·s. This dramatic reduction of 76.6% was statistically significant ($p < 0.05$) from T_2 onwards when compared to the value at T_1., exceeding sample A's reduction from 2209.3

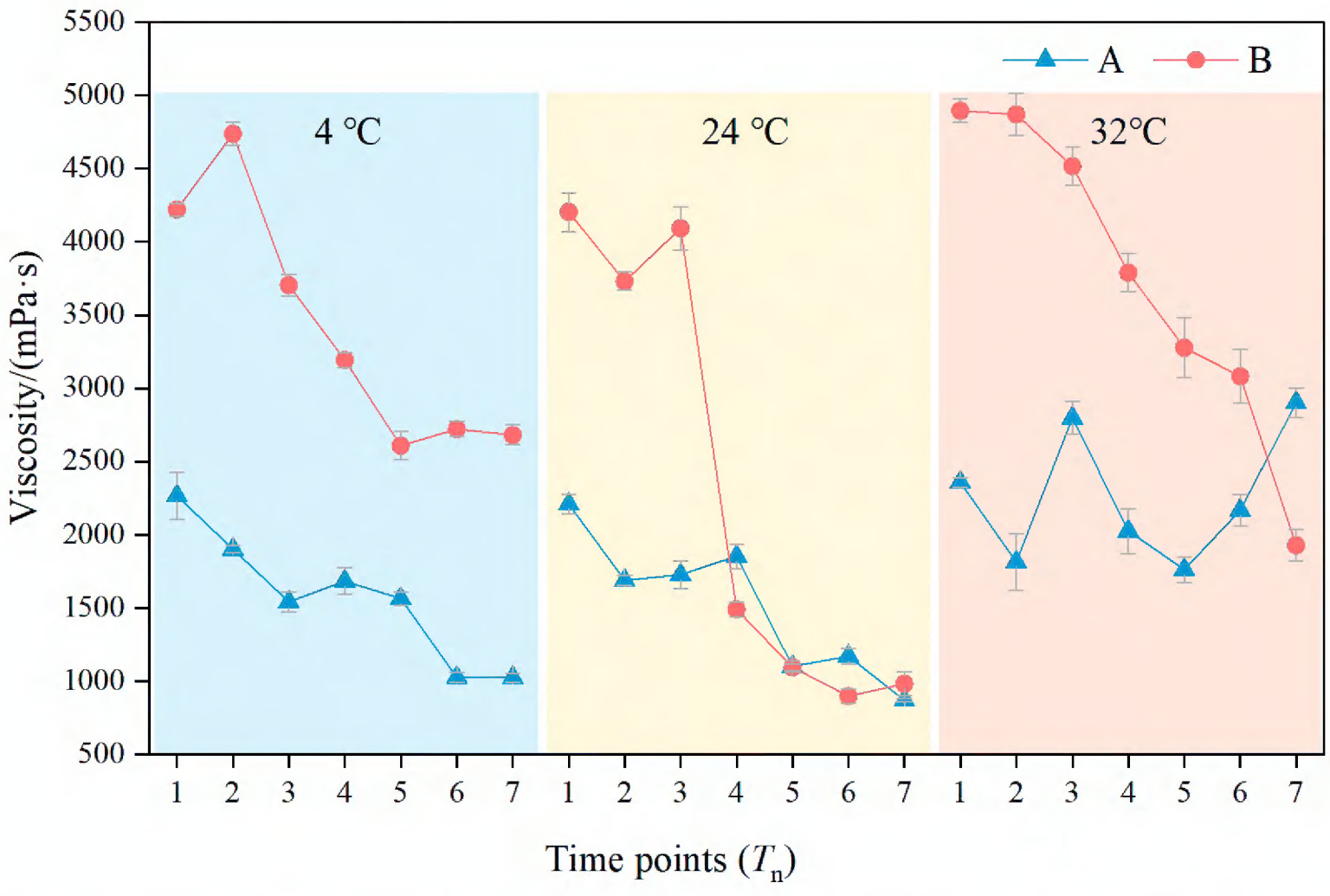


Figure 8. Trend of Viscosity (mPa·s) in expired yogurt under different storage temperatures over time.

to 869.8 mPa·s (60.6%). A notable deviation occurred at 32 °C where sample A's viscosity increased by 23.1%, while sample B decreased sharply by 60.6%.

These divergent trends can be attributed to temperature-dependent microbial and biochemical activities. The larger viscosity loss in sample A at 4 °C may result from cold-induced structural rearrangements, such as casein micelle aggregation and syneresis, exacerbated by its lower initial protein content and weaker gel network (Markoska et al. 2020). At moderate and high temperatures (24–32 °C), microbial metabolism becomes a dominant factor: sample B, potentially containing more thermolabile or proteolytically active strains (e.g., *Bifidobacterium*), may undergo rapid matrix disruption due to enzymatic proteolysis and acid production. The temporary viscosity increase in sample A at 32 °C could reflect initial microbial exopolysaccharide (EPS) synthesis or transient swelling of protein particles, overshadowing gradual breakdown early in storage (Bullard et al. 2018; Wang et al. 2024). Such complex, non-monotonic changes highlight how composition-specific microbial ecology and temperature-driven dynamics collectively dictate rheological evolution in expired yogurt.

Color

Color differences were interpreted according to industry-accepted thresholds based on human visual perception (ASTM D3134-15, 2023). Based on the color difference (ΔE^*) values presented in Table 3, all expired yogurt samples exhibited minimal overall color changes throughout the storage period, as the majority of ΔE^* readings remained below 2.0 across both samples and all temperature conditions. This indicates that the color shifts were largely imperceptible or only slight enough to be detected by trained observers, suggesting good color stability of the products after expiration.

A slight increase in color variability was observed at elevated temperatures, particularly in sample A stored at 32 °C, where ΔE^* values occasionally exceeded 2.0 (e.g., T_6: 2.24), implying minor but measurable color alterations likely induced by temperature-accelerated chemical reactions such as non-enzymatic browning or pigment degradation. Nevertheless, even at 32 °C, the magnitude of ΔE^* remained low overall, and no values approached the threshold of obvious visual difference ($\Delta E^* > 5.0$). Sample B consistently demonstrated superior color stability compared to sample A, with all ΔE^* values remaining below 1.0 under 4 °C and 24 °C storage, further supporting its enhanced resistance to color deterioration.

These results suggest that while temperature exerts a mild influence on color consistency, especially under stressful conditions. The overall color attributes of both yogurt samples were well preserved throughout post-expiration storage.

Table 3. Color difference (ΔE^*) of expired yogurt compared to pre-expiration values under different storage temperatures.

Time points	4 °C		24 °C		32 °C	
	A	B	A	B	A	B
1	0.79	0.62	0.75	0.43	1.88*	1.56*
2	0.62	0.19	1.28*	0.42	1.77*	0.57
3	0.63	0.22	0.70	0.29	0.58	0.42
4	1.02*	0.31	1.62*	0.61	1.82*	0.56
5	0.78	0.49	1.20*	0.62	1.49*	0.64
6	1.10*	0.57	1.03	0.83	2.24*	0.45
7	0.36	0.45	0.60	0.88	1.95*	1.27*

* Denotes changes perceptible to trained observers.

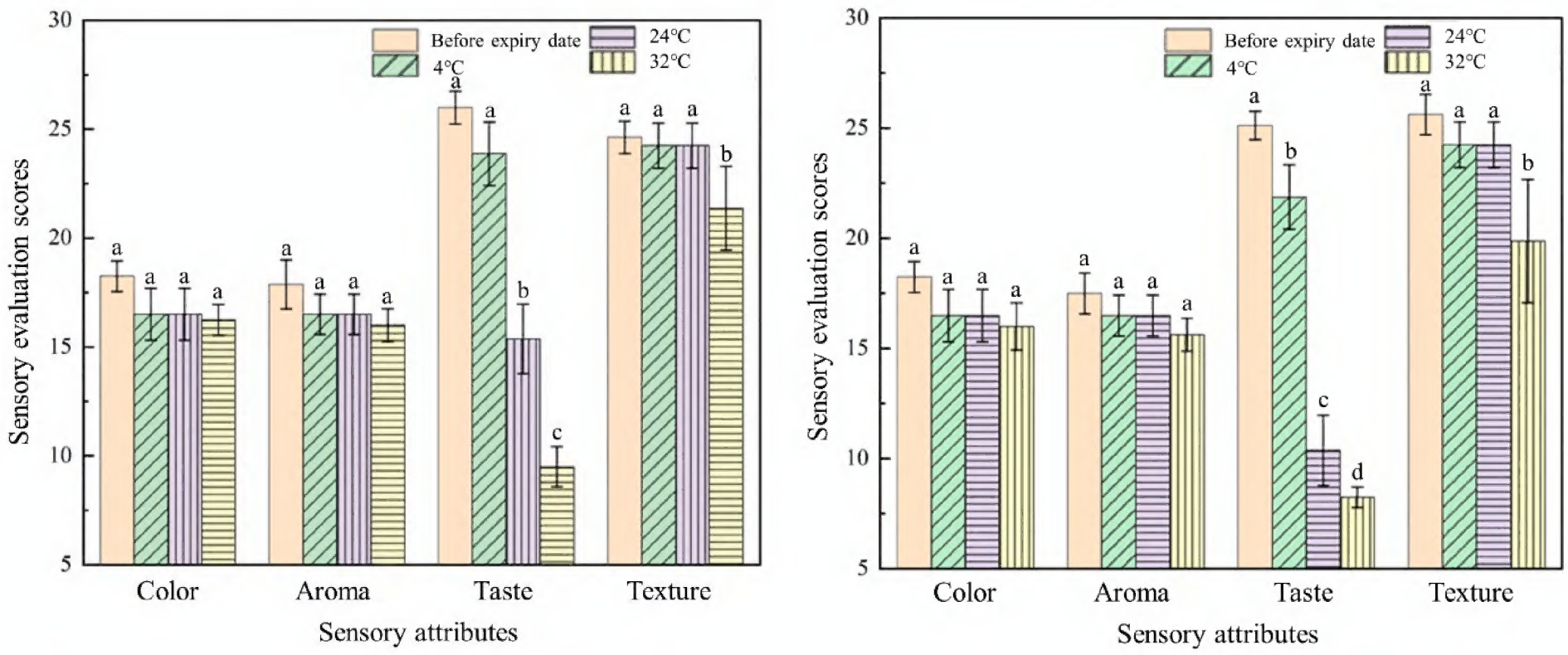
Sensory analysis

To balance tasting risks and sample representativeness, the study selected yogurt samples from the third measurement point (T_3) for sensory evaluation. Although sensory data were collected at a single time point, the observed scores can be contextualized within the longitudinal physicochemical trends. The pronounced deterioration in taste and texture at T_3 ($p < 0.05$), particularly for samples stored at 24 °C and 32 °C, is a direct sensory manifestation of the progressive acidification (Fig. 5), loss of viscosity (Fig. 8), and increased syneresis (Fig. 7) measured throughout the storage period. This correlation allows us to infer that the decline in sensory acceptability was not abrupt but followed a trajectory paralleling the gradual physicochemical degradation. The T_3 time point thus serves as a critical indicator where these cumulative changes had reached a level of clear sensory significance.

As shown in Fig. 9, the sensory profile of sample A exhibited desirable characteristics prior to expiration, including a uniform, milky-white color, a pronounced dairy aroma, a well-balanced sweet-sour taste, and a consistent, smooth texture. However, after exceeding the shelf life and under elevated storage temperatures, the product underwent notable deterioration: the color faded, the dairy aroma diminished, the acidity increased noticeably, and off-odors developed under high-temperature conditions. Whey separation was also observed, contributing to a reduction in overall sensory scores.

A similar declining trend in sensory scores was observed for Yogurt Brand B, though it consistently received higher ratings in flavor and texture compared to Brand A. Notably, samples of Yogurt Brand B stored at 4 °C for 21 days post-expiration showed minimal differences in color, odor, taste, and texture compared to their fresh counterparts, indicating relatively good retention of sensory quality. In contrast, samples stored at ambient (24 °C, 12 days post-expiration) and elevated (32 °C, 3 days post-expiration) temperatures exhibited significant declines in taste scores and overall acceptability.

Overall, both brands demonstrated a gradual decline in sensory quality with prolonged storage post-expiration and increased temperature, manifested as fading color, weakened aroma, deteriorated taste, and coarsened texture. These changes highlight the combined impact of temporal and thermal factors on the deterioration of yogurt quality.



(a) Sensory evaluation of yogurts of sample A

(b) Sensory evaluation of yogurts of sample B

Figure 9. Sensory evaluation of yogurts of sample A (a) and sample B (b) under selected conditions (T_3).

An analysis of the sensory scores reveals that not all attributes contributed equally to the decline in overall acceptability. "Taste" (specifically, the balance between sweetness and sourness) and "Texture" (smoothness and the absence of whey separation) emerged as the primary drivers of consumer rejection post-expiration. The data indicate that the development of excessive sourness (a sharp increase in titratable acidity, as shown in Fig. 5) and the physical breakdown of the gel structure leading to syneresis (quantified as increased whey separation in Fig. 7b) were the most critical factors rendering the products organoleptically unacceptable. This prioritization of taste (sourness) and texture (syneresis) over visual and aromatic changes as the primary drivers of unacceptability is consistent with consumer rejection patterns identified in other studies on dairy product spoilage (e.g., Shin and Lee 2018). Our findings quantitatively confirm that even in the absence of safety concerns, these specific sensory defects are the ultimate determinants of edibility from a consumer perspective, thereby validating the focus on controlling acidification and structural breakdown in product development aimed at reducing waste. In contrast, changes in "Color" and "Aroma," while present, had a comparatively lesser direct impact on the overall acceptability score once the severe taste and texture defects had manifested.

Interplay between microbiological, physicochemical, and sensory parameters

The longitudinal design of this study allows for an examination of the complex interplay between the various measured parameters, providing a mechanistic understanding of yogurt deterioration beyond the expiration date.

(1) Microbiological activity as the primary driver: the dynamics of the lactic acid bacteria (LAB) population were fundamentally linked to most physicochemical changes. The significant increase in titratable acidity at elevated temperatures (Fig. 5) was a direct consequence of sustained LAB metabolic activity, consuming lactose and producing lactic acid. This relationship is clearly demonstrated by the concurrent rapid decline in reducing sugar content (Fig. 4) and the surge in acidity, particularly in Sample A at 32 °C.

(2) Consequences of acidification on physical structure: the drop in pH due to acidification profoundly affected the yogurt's physical properties. The casein micelle network, which is stable at the initial pH of yogurt (~4.5), becomes increasingly unstable and contracts as acidity rises, leading to syneresis. This is evidenced by the strong negative correlation between the rising titratable acidity (Fig. 5) and the declining water-holding capacity (WHC) and increasing whey separation (Fig. 7) in Sample A at 24 °C and 32 °C. The aggregation and contraction of the protein matrix also directly contributed to the substantial loss of viscosity (Fig. 8), as the gel structure lost its ability to entrap water and maintain its three-dimensional integrity.

(3) Structural breakdown and oxidative stability: the physical breakdown of the gel, indicated by increased whey separation and reduced turbidity, may have accelerated the decline in antioxidant activity (Fig. 3). The expulsion of whey, which contains water-soluble antioxidants, and the increased exposure of the matrix to oxygen due to structural collapse could have promoted oxidative reactions, leading to the degradation of bioactive compounds.

(4) (From instrumental to sensory failure: ultimately, these interconnected microbiological and physicochemical changes culminated in sensory rejection. The excessive sourness perceived by the panelists (Fig. 9) is a direct sensory translation of the high titratable acidity. The gritty, coarse texture and visible whey separation noted in the sensory evaluation are

the human-perceivable equivalents of the measured losses in viscosity, WHC, and increased syneresis. This clear pathway—from microbial metabolism to acid production, to structural degradation, and finally to sensory unacceptability—explains why "taste" and "texture" were the primary drivers of overall unacceptability, while color changes (which were minimal, Table 3) played a lesser role.

Conclusion

This study comprehensively evaluated the microbiological safety, physicochemical properties, and sensory quality of two commercially available yogurt samples (A and B) under different storage temperatures (4 °C, 24 °C, and 32 °C) over a post-expiration period of up to 49 days. The results indicate that both products remained microbiologically safe throughout the study, with no detection of Enterobacteriaceae, *Salmonella*, or *Staphylococcus aureus*, and yeast or mold count within the regulatory limit. LAB count remained above 1×10^6 CFU/mL in all conditions, complying with regulatory requirements. Key findings regarding edibility and quality evolution include:

At 4 °C, both yogurts maintained acceptable sensory and physicochemical qualities for an extended period. Sample B remained comparable to fresh product in color, aroma, taste, and texture for up to 21 days post-expiration, while sample A showed more noticeable changes in viscosity and texture after 14 days. This extended edibility window suggests that proper refrigeration could significantly reduce unnecessary yogurt disposal, contributing to household food waste reduction.

At 24 °C, significant quality degradation was observed after 12 days, particularly in taste and texture, with sample A developing pronounced sourness and whey separation. Sample B demonstrated better stability but still exhibited reduced acceptability beyond this point. These findings provide clear guidance for consumers to distinguish between still-edible and compromised products, preventing premature discard of yogurt that remains safe for consumption.

At 32 °C both products deteriorated rapidly. Sensory and physicochemical properties declined markedly within 3 days, with off-odors, significant acidification, and structural collapse making them organoleptically unacceptable. The rapid spoilage at elevated temperatures underscores the importance of consistent refrigeration to extend yogurt's edible life and minimize waste.

Sample B consistently outperformed sample A in overall stability, likely due to its higher protein content, optimized stabilizer blend, and the presence of *Bifidobacterium animalis* subsp. *lactis*, which moderated acid production and enhanced structural integrity. This demonstrates how product formulation innovations can extend practical shelf life, offering manufacturers strategies to develop products with reduced waste potential.

Implications for labeling policies

Our findings challenge the uniform "one date fits all" approach to yogurt expiration labeling, offering a scientific basis for waste reduction through coordinated action by industry and policymakers.

For regulatory bodies, this study supports a transition to a dual-labeling system that distinguishes between "Best Before" (indicating quality degradation) and "Use By" (reserving for safety-critical products). Yogurt stored at 4 °C clearly belongs to the former category. Furthermore, evidence-based date setting should be established, where products with demonstrated stability—such as those containing specific probiotics (e.g., Bifidobacterium animalis subsp. lactis), higher protein, or optimized stabilizers—could qualify for extended "Best Before" periods based on standardized testing.

For the dairy industry, this research highlights that strategic formulation—using protective probiotics and structural enhancers—can inherently extend practical shelf life and reduce waste. Concurrently, improved consumer communication through clear on-pack messaging (e.g., "Keep Refrigerated – Trust Your Senses") can empower consumers to rely on sensory evaluation rather than fixed dates.

However, generalizing these findings requires caution. Post-expiration behavior depends heavily on product-specific factors, including microbial strains, stabilizers, and composition. Extrapolation to non-dairy yogurts is particularly complex due to their distinct raw materials, protein structures, and microbial ecologies, which may involve different spoilage patterns and risks. Therefore, policy or formulation adjustments based on this model require validation across specific product categories.

Collectively, implementing these evidence-based strategies can transform date labeling from a driver of waste into a tool for its reduction, aligning economic, environmental, and food security goals.

Limitations and future research

This study has several limitations, including the use of constant temperature conditions that do not fully reflect real-world storage variability, a strategic but limited sensory evaluation protocol for safety reasons, and a focus on two specific commercial products which limits broad generalizability. To build upon this work and address these constraints, future research should pursue several key avenues. Investigations should employ dynamic temperature scenarios to simulate realistic supply chain and household storage fluctuations, providing more practical shelf-life predictions. The application of advanced analytical techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS) for volatile profiling and High-Performance Liquid Chromatography (HPLC) for detailed metabolite tracking, would offer a deeper, mechanistic understanding of spoilage beyond the parameters measured here.

Furthermore, expanding the product matrix to include a wider array of yogurt types—particularly diverse non-dairy alternatives with fundamentally different compositions and microbial ecologies—is essential for validating the universal applicability of the current findings. Finally, integrating larger-scale consumer studies is crucial to bridge the gap between scientific metrics and real-world practice, effectively validating sensory thresholds identified by trained panels while also exploring the socio-psychological factors that ultimately drive consumption or disposal decisions. Collectively, these research directions would significantly enhance the generalizability, mechanistic insight, and practical impact of future strategies aimed at reducing food waste.

Despite these limitations, this study provides practical guidance for consumers and retailers: yogurt stored consistently at 4 °C may remain edible for 2–3 weeks post-expiration, while products exposed to higher temperatures should be discarded within a few days. Ultimately, sensory evaluation—particularly off-odors, abnormal texture, and excessive sourness—should be the final determinant of edibility.

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